

be critical for proper biological function, and serve as markers for various disease states. To study the effects of these important mutations, we have prepared constructs of the caveolin protein that encompass the transmembrane domain. Both Caveolin(99-137) and caveolin(122-142) were over-expressed and isotopically labeled in *E.coli*, purified to homogeneity, and incorporated into dodecylphosphocholine micelles. The three-dimensional structure of the constructs will be revealed using a combination of circular dichroism and NMR spectroscopy. Moreover, comparison of the wild-type structure with that of the constructs containing the proline mutations will give valuable insight into the unusual nature of the caveolin-1 transmembrane domain.

#### 2082-Pos Board B68

##### Reconstitution and Topological Analysis of Caveolin-1 in Phospholipid Vesicles

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One of the greatest challenges of working with membrane proteins is their reconstitution into native bilayers. The extreme insolubility of integral membrane proteins renders them incompatible with most common reconstitution methods. It has been shown that perfluorooctanoic acid (PFOA) is a very favorable detergent for highly hydrophobic peptides and proteins. Therefore, a method has been developed using PFOA to reconstitute proteins into egg lecithin unilamellar vesicles. Using dynamic light scattering and electron microscopy, vesicles generated by this method are spherical and have a hydrodynamic radius of 14 nm. In addition, the entrapment of glucose by the vesicles demonstrates that they are indeed bilayered and have a hollow center. The membrane interacting domain of caveolin-1 has been incorporated into these vesicles. Using the Stern-Volmer analysis methodology, fluorescence quenching studies of the tryptophan residues in the caveolin-1 membrane interacting domain has given qualitative structural information of how caveolin-1 interacts with the lipid bilayer. In addition, the topology of caveolin-1 has been explored using proteolysis. The construct contains one enterokinase cleavage site at the N- and C- termini. Both termini were cleaved with the enterokinase enzyme confirming the “horseshoe” orientation of the caveolin-1 membrane interacting domain in the bilayer. We believe these studies will provide further insight into the caveolin-1 transmembrane domain structure in a lipid bilayer. These experiments begin to address the long-standing uncertainties surrounding the topology of the caveolin protein.

## Protein Assemblies

#### 2083-Pos Board B69

##### Automated Prediction of Protein-Protein Association Rate Constants

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The association of two proteins generally occurs in two steps [1]. The first is the formation of a transient complex, in which the two proteins have near-native separation and relative orientation. In the subsequent step, the proteins undergo conformational rearrangement to form the native short-range interactions. Orientational restraints of the transient complex limit its formation to a “basal” rate constant  $\sim 10^5 \text{ M}^{-1}\text{s}^{-1}$ , but long-range electrostatic attraction can enhance the rate by three to four orders of magnitude [1]. In our recently developed transient-complex theory [1], the electrostatically enhanced association rate is predicted as  $k_a = k_{a0} \exp(-\langle U_{el} \rangle^*/k_B T)$ , where  $k_{a0}$  is the basal rate constant and  $\langle U_{el} \rangle^*$  is the average electrostatic interaction energy between the proteins in the transient complex. The predictive power of the transient-complex theory has been demonstrated over a large number of protein-protein and protein-RNA complexes [1-3]. To make our method widely accessible for predicting protein association rate constants, here we developed it into a web server. The “TransComp” server is available at <http://pipe.sc.fsu.edu/transcomp/>, with the structure of the native complex formed by two proteins as input. The generation of the transient complex and the calculation of  $k_{a0}$  and  $\langle U_{el} \rangle^*$  are done automatically. Test run of the server on 14 protein-protein complexes was carried out to demonstrate its capability. Association rate constants calculated by the server for these complexes agree well with experimental data, which range from  $5.5 \times 10^4$  to  $2.4 \times 10^8 \text{ M}^{-1}\text{s}^{-1}$ . We expect that the TransComp server will become a valuable tool for kinetic characterization of protein-protein and protein-nucleic acid association.

[1] R. Alsallaq and H.-X. Zhou (2008) *Proteins* 71, 320-335.

[2] S.B. Qin and H.-X. Zhou (2008) *J. Phys. Chem. B* 112, 5955-5960.

[3] S.B. Qin and H.-X. Zhou (2009) *Proc. Natl. Acad. Sci. USA* 106, 6974-7979.

#### 2084-Pos Board B70

##### Prediction of Protein-Protein Interactions at Genome Scale

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Construction of the structural protein interaction network is of crucial importance since it is a prerequisite for understanding how the proteome, and thus the cell, function. Yet, predicting, on the proteome scale, which proteins interact and how they interact is a daunting task. Structural predictions of protein interactions are frequently carried out via ‘docking’. However, in the absence of additional biochemical data, docking is challenging on the proteome scale because there are many favorable ways for proteins to interact. An alternative strategy is knowledge-based, using a protein-protein interface dataset. Using such dataset is efficient because the number of architectures, in single chain proteins and in protein-protein interfaces is limited in nature, and structurally different protein pairs can use the same (preferred) binding architectures. This suggests that using structural alignment of each side of known interfaces against the entire surfaces of all monomers can predict protein associations: a protein whose surface matches one side of the interface can bind a protein whose surface matches the complementary side. Yet, on their own knowledge-based methods may not be sufficient for proteome modeling because they disregard flexibility and energetics. Here, for the first time, we combine the two methods, leading to a powerful combinatorial multi-scale strategy to predict functional associations of the proteome. As examples, we present the tumor suppressor protein p53 interaction network, focusing on the nucleotide excision repair and cyclin dependent kinase subsystems. The validated examples demonstrate the power of this strategy. New interactions are also predicted for NFκB, p27 and Skp2.

#### 2085-Pos Board B71

##### Monte Carlo Study of the Molecular Mechanisms of S-Layer Protein Self-Assembly

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Self-assembly is one of nature’s strategies to organize matter on the large scale and thereby create order from disorder. The process is ubiquitous for a great variety of biological molecules. Proteins, however, tend to stay soluble in solution or to aggregate into various structures rather than self-assemble into defined patterns. This is because of their complex structure exhibiting different conformations and a close-knit relationship between structure and function. Aggregation into three-dimensional composites thus generally leads to a loss of functionality. However, S(urface)-layer proteins, which constitute the crystallized outer most cell envelope of a great variety of bacterial cells, represent a remarkable exception to this general trend. The crystallization of this kind of proteins facilitates their function rather than forming a nonfunctional state. Using a combination of structural information and a Monte Carlo method with a coarse-grained model, we have studied the functional protein self-assembly into S-layers. The molecular mechanisms guiding the self-assembly of proteins into functional or pathogenic large-scale structures can be only understood by studying the correlation between the structural details of the monomer and the eventual mesoscopic morphologies. Among the myriad structural details of protein monomers, we seek to identify the most crucial set of structural features necessary for the spontaneous selection of desired morphologies. We discover that only few and mainly hydrophobic amino acids, located on the surface of the monomer, are responsible for the formation of a highly ordered anisotropic protein lattice. In addition to elucidating the molecular mechanisms and explaining experimental findings, the present work offers a tool, which is chemical enough to capture details of primary sequences and coarse-grained enough to explore morphological structures with thousands of protein monomers, to promulgate design rules for spontaneous formation of specific protein assemblies.

#### 2086-Pos Board B72

##### The Importance of Cell Lysis Methods in Measuring Proteasome Activity

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Proteasome dysfunction has been implicated in a wide range of diseases, including neurodegenerative disorders such as Alzheimer’s or Parkinson’s diseases, as well as cardiomyopathies and diabetes. The role of the proteasome is critical to the normal functioning of the body’s degradation pathways. Numerous methods of isolating and measuring proteasome activity in cell lysates are currently being used by different groups. In particular, the homogenization and lysis buffers vary considerably between different laboratories. In this study, we investigated how different cell lysis procedures and buffers affect both 20S and 26S proteasome proteolytic activity. Our results suggest that the inclusion of glycerol in the lysis buffer is important for optimal 20S proteasome activity. We also found that in comparison to cell lysis buffers